



Title: IDENTIFICATION OF THE GENE CAUSING THE MOUSE SCURFY PHENOTYPE AND ITS HUMAN ORTHOLOG

Express Mail No. EV336613357US

Inventor(s): Mary E. Brunkow et al. Serial No. 09/696,867 Docket No. 240083.501D6

MOUSE *Fkh^{sf}* cDNA SEQUENCE

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1  GCTGATCCCC CTCTAGCAGT CCACTTCACC AAGGTGAGCG AGTGTCCCTG
51  CTCTCCCCCA CCAGACACAG CTCTGCTGGC GAAAGTGGCA GAGAGGTATT
101 GAGGGTGGGT GTCAGGAGCC CACCAGTACA GCTGGAAACA CCCAGCCACT
151 CCAGCTCCCG GCAACTTCTC CTGACTCTGC CTTCAGACGA GACTTGGAAG
201 ACAGTCACAT CTCAGCAGCT CCTCTGCCGT TATCCAGCCT GCCTCTGACA
251 AGAACCCAAT GCCCAACCCT AGGCCAGCCA AGCCTATGGC TCCTTCCTTG
301 GCCCTTGGCC CATCCCCAGG AGTCTTGCCA AGCTGGAAGA CTGCACCCAA
351 GGGCTCAGAA CTTCTAGGGA CCAGGGGCTC TGGGGGACCC TTCCAAGGTC
401 GGGACCTGCG AAGTGGGGCC CACACCTCTT CTTCTTGAA CCCCCTGCCA
451 CCATCCCAGC TGCAGCTGCC TACAGTGCCC CTAGTCATGG TGGCACCGTC
501 TGGGGCCCGA CTAGGTCCCT CACCCACCT ACAGGCCCTT CTCCAGGACA
551 GACCACACTT CATGCATCAG CTCTCCACTG TGGATGCCCA TGCCAGACC
601 CCTGTGCTCC AAGTGCCTCC ACTGGACAAC CCAGCCATGA TCAGCCTCCC
651 ACCACCTTCT GCTGCCACTG GGGTCTTCTC CCTCAAGGCC CGGCCTGGCC
701 TGCCACCTGG GATCAATGTG GCCAGTCTGG AATGGGTGTC CAGGGAGCCA
751 GCTCTACTCT GCACCTTCCC ACGCTCGGGT ACACCCAGGA AAGACAGCAA
801 CCTTTTGGCT GCACCCCAAG GATCCTACCC ACTGCTGGCA AATGGAGTCT
851 GCAAGTGGCC TGGTTGTGAG AAGGTCTTCG AGGAGCCAGA AGAGTTTCTC
901 AAGCACTGCC AAGCAGATCA TCTCCTGGAT GAGAAAGGCA AGGCCAGTG
951 CCTCCTCCAG AGAGAAGTGG TGCAGTCTCT GGAGCAGCAG CTGGAGCTGG
1001 AAAAGGAGAA GCTGGGAGCT ATGCAGGCCC ACCTGGCTGG GAAGATGGCG
1051 CTGGCCAAGG CTCCATCTGT GGCCTCAATG GACAAGAGCT CTTGCTGCAT
1101 CGTAGCCACC AGTACTCAGG GCAGTGTGCT CCCGGCCTGG TCTGCTCCTC
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Fig. 1A



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1151 GGGAGGCTCC AGACGGCGGC CTGTTTGCAG TGCGGAGGCA CCTCTGGGGA
1201 AGCCATGGCA ATAGTTCCTT CCCAGAGTTC TTCCACAACA TGGACTACTT
1251 CAAGTACCAC AATATGCGAC CCCCTTTCAC CTATGCCACC CTTATCCGAT
1301 GGGCCATCCT GGAAGCCCCG GAGAGGCAGA GGACACTCAA TGAAATCTAC
1351 CATTGGTTTA CTCGCATGTT CGCCTACTTC AGAAACCACC CCGCCACCTG
1401 GAAGAATGCC ATCCGCCACA ACCTGAGCCT GCACAAGTGC TTTGTGCGAG
1451 TGGAGAGCGA GAAGGGAGCA GTGTGGACCG TAGATGAATT TGAGTTTCGC
1501 AAGAAGAGGA GCCAACGCCC CAACAAGTGC TCCAATCCCT GCCCTTGACC
1551 TCAAAACCAA GAAAAGGTGG GCGGGGGAGG GGGCCAAAC CATGAGACTG
1601 AGGCTGTGGG GGCAAGGAGG CAAGTCCTAC GTGTACCTAT GGAAACCGGG
1651 CGATGATGTG CCTGCTATCA GGGCCTCTGC TCCCTATCTA GCTGCCCTCC
1701 TAGATCATAT CATCTGCCTT ACAGCTGAGA GGGGTGCCAA TCCCAGCCTA
1751 GCCCCTAGTT CCAACCTAGC CCCAAGATGA ACTTTCCAGT CAAAGAGCCC
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1851 ACAGACACCA TCCTAATATT TACTCAACCC AAACCCTAAA ACATGAAGAG
1901 CCTGCCTTGG TACATTCGTG AACTTTCAAA GTTAGTCATG CAGTCACACA
1951 TGA CTGCAGT CCTACTGACT CACACCCCAA AGCACTCACC CACAACATCT
2001 GGAACCACGG GCACTATCAC ACATAGGTGT ATATACAGAC CCTTACACAG
2051 CAACAGCACT GGAACCTTCA CAATTACATC CCCCCAAACC ACACAGGCAT
2101 AACTGATCAT ACGCAGCCTC AAGCAATGCC CAAAATACAA GTCAGACACA
2151 GCTTGTGAGA
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Fig. 1B



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MOUSE Fkh^{sf} PROTEIN SEQUENCE

```
1  MPNPRPAKPM APSLALGPSP GVLPSWKTAP KGSELLGTRG SGGPFQGRDL
51  RSGAHTSSSL NPLPPSQLQL PTVPLVMVAP SGARLGPSPH LQALLQDRPH
101 FMHQLSTVDA HAQTPVLQVR PLDNPAMISL PPPSAATGVF SLKARPGGLPP
151 GINVASLEWV SREPALLCTF PRSGTPRKDS NLLAAPQGSY PLLANGVCKW
201 PGCEKVFEED EEFLKHCQAD HLLDEKGKAQ CLLQREVVSQ LEQQLELEKE
251 KLGAMQAHLA GKMALAKAPS VASMDKSSCC IVATSTQGSV LPAWSAPREA
301 PDGGLFAVRR HLWGSHGNSS FPEFFHNMDY FKYHNMRPPF TYATLIRWAI
351 LEAPERQRTL NEIYHWFTRM FAYFRNHPAT WKNAIRHNLS LHKCFVRVES
401 EKGAVWTVDE FEFRRKRSQR PNKCSNPCP*
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Fig. 2



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HUMAN *FKH^{sf}* cDNA Sequence

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1  GCACACACTC ATCGAAAAAA ATTTGGATTA TTAGAAGAGA GAGGTCTGCG
51  GCTTCCACAC CGTACAGCGT GGTTTTTCTT CTCGGTATAA AAGCAAAGTT
101 GTTTTTGATA CGTGACAGTT TCCCACAAGC CAGGCTGATC CTTTTCTGTC
151 AGTCCACTTC ACCAAGCCTG CCCTTGGACA AGGACCCGAT GCCCAACCCC
201 AGGCCTGGCA AGCCCTCGGC CCCTTCCTTG GCCCTTGGCC CATCCCCAGG
251 AGCCTCGCCC AGCTGGAGGG CTGCACCCAA AGCCTCAGAC CTGCTGGGGG
301 CCCGGGGCCC AGGGGGAACC TTCCAGGGCC GAGATCTTCG AGGCGGGGCC
351 CATGCCTCCT CTTCTTCCTT GAACCCCATG CCACCATCGC AGCTGCAGCT
401 GCCCACACTG CCCCTAGTCA TGGTGGCACC CTCCGGGGCA CGGCTGGGCC
451 CCTTGCCCCA CTTACAGGCA CTCCTCCAGG ACAGGCCACA TTTTCATGAC
501 CAGCTCTCAA CGGTGGATGC CCACGCCCGG ACCCCTGTGC TGCAGGTGCA
551 CCCCTGGAG AGCCAGCCA TGATCAGCCT CACACCACCC ACCACCGCCA
601 CTGGGGTCTT CTCCCTCAAG GCCCGGCCTG GCCTCCCACC TGGGATCAAC
651 GTGGCCAGCC TGGAAATGGT GTCCAGGGAG CCGGCACTGC TCTGCACCTT
701 CCCAAATCCC AGTGACCCCA GGAAGGACAG CACCCTTTCG GCTGTGCCCC
751 AGAGCTCCTA CCCACTGCTG GCAAATGGTG TCTGCAAGTG GCCCGGATGT
801 GAGAAGGTCT TCGAAGAGCC AGAGGACTTC CTCAAGCACT GCCAGGCGGA
851 CCATCTTCTG GATGAGAAGG GCAGGGCACA ATGTCTCCTC CAGAGAGAGA
901 TGGTACAGTC TCTGGAGCAG CAGCTGGTGC TGGAGAAGGA GAAGCTGAGT
951 GCCATGCAGG CCCACCTGGC TGGGAAAATG GCACTGACCA AGGCTTCATC
1001 TGTGGCATCA TCCGACAAGG GCTCCTGCTG CATCGTAGCT GCTGGCAGCC
1051 AAGGCCCTGT CGTCCCAGCC TGGTCTGGCC CCCGGGAGGC CCCTGACAGC
1101 CTGTTTGCTG TCCGGAGGCA CCTGTGGGGT AGCCATGGAA ACAGCACATT
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Fig. 3A



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1151 CCCAGAGTTC CTCCACAACA TGGACTACTT CAAGTTCCAC AACATGCGAC
1201 CCCCTTTTAC CTACGCCACG CTCATCCGCT GGGCCATCCT GGAGGCTCCA
1251 GAGAAGCAGC GGACACTCAA TGAGATCTAC CACTGGTTCA CACGCATGTT
1301 TGCCTTCTTC AGAAACCATC CTGCCACCTG GAAGAACGCC ATCCGCCACA
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1401 GTGTGGACCG TGGATGAGCT GGAGTTCCGC AAGAAACGGA GCCAGAGGCC
1451 CAGCAGGTGT TCCAACCCTA CACCTGGCCC CTGACCTCAA GATCAAGGAA
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1651 CCTCCCCAT CATATCCTTT GCCCAAGGC TGCTCAGAGG GGCCCCGGTC
1701 CTGGCCCCAG CCCCCACCTC CGCCCCAGAC ACACCCCCA GTCGAGCCCT
1751 GCAGCCAAAC AGAGCCTTCA CAACCAGCCA CACAGAGCCT GCCTCAGCTG
1801 CTCGCACAGA TTACTTCAGG GCTGGAAAAG TCACACAGAC ACACAAAATG
1851 TCACAATCCT GTCCCTCAC

Fig. 3B



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1 MPNPRPGKPS APSLALGPSP GASPSWRAAP KASDLLGARG PGGTFQGRDL
51 RGGAHASSSS LNPMPPSQLQ LPTLPLVMVA PSGARLGPLP HLQALLQDRP
101 HFMHQLSTVD AHARTPVLQV HPLESPAMIS LTPPTTATGV FSLKARPGLP
151 PGINVASLEW VSREPALLCT FPNPSAPRKD STLSAVPQSS YPLLANGVCK
201 WPGCEKVFEED PEDFLKHCQA DHLLDEKGRA QCLLQREMVD SLEQQLVLEK
251 EKLSAMQAHLE AGKMALTKAS SVASSDKGSC CIVAAGSQGP VVPAWSGPRE
301 APDSLFAVRR HLWGSHGNST FPEFLHNMDY FKFHNMRPPF TYATLIRWAI
351 LEAPEKQRTL NEIYHWFTRM FAFFRNHPAT WKNAIRHNLS LHKCFVRVES
401 EKGAVWTVDE LEFRKKRSQR PSRCSNPTPG P*

Fig. 4



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Vector for generation of FKH^{sf} Transgenic mice



Fig. 5



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FKHsf Transgene corrects the defect in
scurfy animals

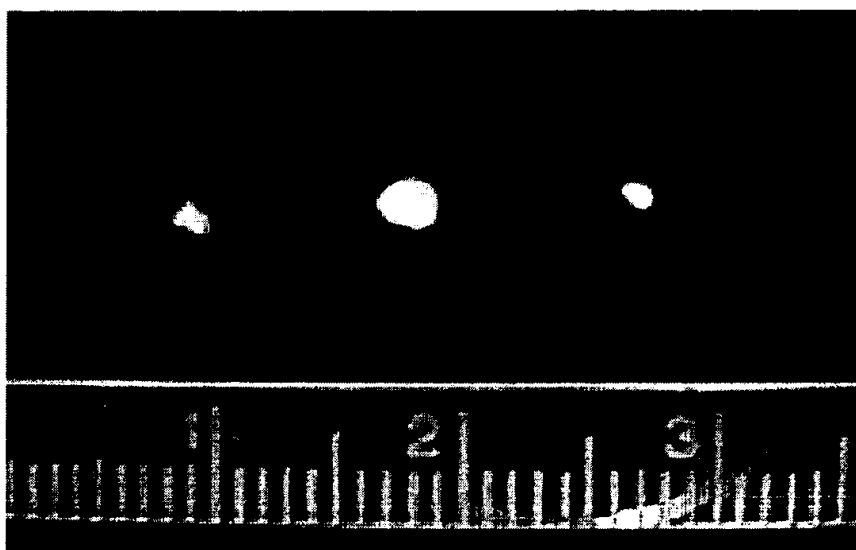


Fig. 6



FKHsf tg mice have reduce lymph node cells
compared to normal cells

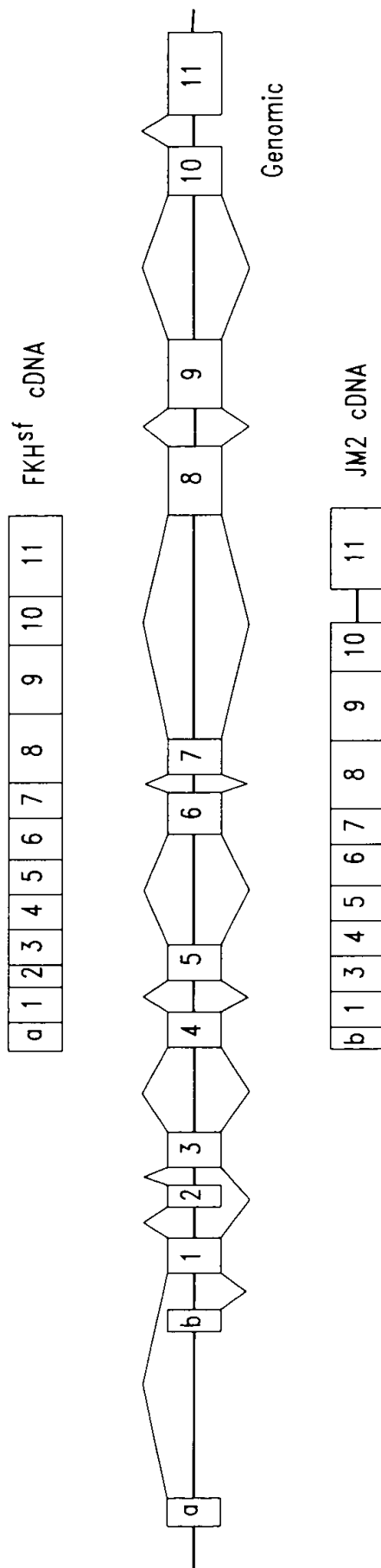
Cell number	Mouse genotype		
	Normal	Scurfy	Transgenic
Cells / LN	0.92	1.97	0.29
Cells / Thymus	0.76	0.54	0.76

Fig. 7

FKHsf transgenic mice respond poorly to in vitro stimulation

Proliferation	Mouse genotype		
	Normal	Scurfy	Transgenic
No stimulation	778	23488	596
Anti-CD3+Anti-CD28	22932	225981	9106

Fig. 8



Comparison of FKH^{sf} and JM2 cDNAs. Exon/intron structure is shown (Genomic) as open rectangles (exons) joined by heavy horizontal lines (introns). Coding exons are numbered 1-11 as determined by sequence analysis of FKH^{sf} cDNA; non-coding 5' exons are labelled *a* and *b*. The FKH^{sf}-specific and JM2-specific splicing patterns and resulting cDNAs are indicated above and below the genomic structure, respectively.

Fig. 9



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Human FKH ^{sf}				Mouse Fkh ^{sf}			
N-terminal	ZNF	Mid	Forkhead	N-terminal	ZNF	Mid	Forkhead
83.4%	95.8%	82.8%	96.4%				

Human and mouse FKH^{sf} proteins are highly conserved.

Fig. 10